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THE ACTION OF β-ADRENOCEPTOR AGONISTS ON ACID SECRETION BY THE RAT ISOLATED STOMACH

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SUMMARY

1. The action of β-adrenoceptor agonists on acid secretion by an immature rat isolated stomach preparation has been studied.

2. Isoprenaline, salbutamol, salmefamol, adrenaline and noradrenaline all stimulated acid output over a concentration range of 2×10^{-7} M–10^{-5} M.

3. These responses were antagonized by propranolol (2×10^{-5} M), pindolol and timolol (10^{-6} M).

4. The antagonism of isoprenaline and salmefamol by propranolol was consistent with competitive inhibition.

5. Selective β-adrenoceptor antagonists (practolol, atenolol, butoxamine and ICI 118 551) caused significant inhibition of noradrenaline-stimulated secretion but not of that due to the other agonists.

6. An adult rat isolated mucosa preparation responded to adrenaline in a similar manner to the immature stomach preparation.

7. Acid secretion stimulated by β-adrenoceptor agonists was not antagonized by atropine (10^{-5} M), metiamide (10^{-4} M) or prostaglandin E_2 (10^{-5} M). The concentrations of these three antagonists caused marked inhibition of the responses to submaximal concentrations of bethanechol, histamine and pentagastrin respectively.

8. The results are discussed in relation to the possible mechanisms of action of β-adrenoceptor stimulation of acid secretion: it is concluded that the response is probably mediated by β-receptors on the parietal cell.

INTRODUCTION

In the preceding paper (Canfield & Price, 1981) we have shown that isoprenaline stimulates acid secretion by the immature rat isolated stomach preparation, in contrast to the inhibitory effect of this and similar compounds *in vivo*. In this paper we present the results of a more extensive investigation of the effects of β-adrenoceptor agonists on acid secretion *in vitro* and the action of both adrenoceptor antagonists

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and other established inhibitors of acid secretion on these responses. The results are discussed in relation to the possible mechanisms of β-adrenoceptor-stimulated acid secretion in the isolated stomach. Some of this work has been briefly communicated to the Physiological and Pharmacological Societies (Canfield, Curwain, King & Price, 1978; Canfield, Curwain & Price, 1978; Canfield & Price, 1980; Canfield, Price & Spencer, 1980).

METHODS

Isolated stomach preparations

Isolated stomach preparations were set up as detailed in the preceding paper (Canfield & Price, 1981). All drugs were added to the serosal bathing fluid and the unbuffered mucosal solution was replaced at 15 min intervals for estimation of acid secretion by titration. The response to drug addition in each stomach is expressed as the secretory ratio (R), where

$$R = \frac{\text{Average plateau response to drug}}{\text{Average spontaneous secretion}},$$

as described in the preceding paper. Some absolute values (response − spontaneous secretion, μmol H^+ cm^{-2} h^{-1}) are included in brackets in the text to facilitate comparison of the data with other published work using isolated stomachs.

Adult mucosa

Stomachs were removed from Wistar rats weighing 250–350 g and pinned out, mucosal surface down, on a moist cork board. Mucosal saline was injected between the mucosal and muscle layers to separate them and the muscle layer then dissected away. The isolated mucosal preparation was then treated in the same manner as the immature stomach.

Drugs

The following drugs were used: histamine acid phosphate, isoprenaline sulphate (MacCarthys); salbutamol, salmefamol (Glaxo Research); adrenaline (Antigen); noradrenaline (Winthrop); bethanechol chloride (Glenwood Laboratories); pentagastrin, propranolol, practolol, atenolol, ICI 118 551 (ICI Ltd); phentolamine (Ciba); butoxamine (Burroughs Wellcome); pindolol (Sandoz); timolol (Merck, Sharp & Dohme); atropine sulphate (BDH Ltd); metiamide (S.K. & F. Ltd); and prostaglandin E_2 (Upjohn).

Statistics

Responses in the presence of an antagonist were compared with control stomachs using the Mann–Witney U test as in the preceding paper. Tests were performed on the secretory ratios.

RESULTS

Concentration–response curves

Fig. 1*A* shows the log concentration–response curves for the β_2-selective agonists salmefamol and salbutamol and also the isoprenaline curve from the preceding paper for comparison (Canfield & Price, 1981), whilst Fig. 1*B* shows the results with the two naturally occurring catecholamines. The maximum responses and slopes of the relationships are similar for all five drugs. The mean maximum secretory ratios with their S.E. of the means were: salmefamol $1{\cdot}90 \pm 0{\cdot}09$ ($n = 21$) ($3{\cdot}84 \pm 0{\cdot}28$ μmol H^+ cm^{-2} h^{-1}), salbutamol $2{\cdot}10 \pm 0{\cdot}21$ ($n = 5$) ($3{\cdot}61 \pm 0{\cdot}28$ μmol H^+ cm^{-2} h^{-1}), adrenaline $2{\cdot}22 \pm 0{\cdot}08$ ($n = 7$) ($3{\cdot}02 \pm 0{\cdot}44$ μmol H^+ cm^{-2} h^{-1}) and noradrenaline $2{\cdot}03 \pm 0{\cdot}13$ ($n = 6$) ($3{\cdot}19 \pm 0{\cdot}38$ μmol H^+ cm^{-2} h^{-1}). Isoprenaline was the most potent agonist and noradrenaline the least, but no uptake inhibitors were used and this may partly account for the noradrenaline results. Neither β_2-selective agonist was as potent as isoprenaline or adrenaline.

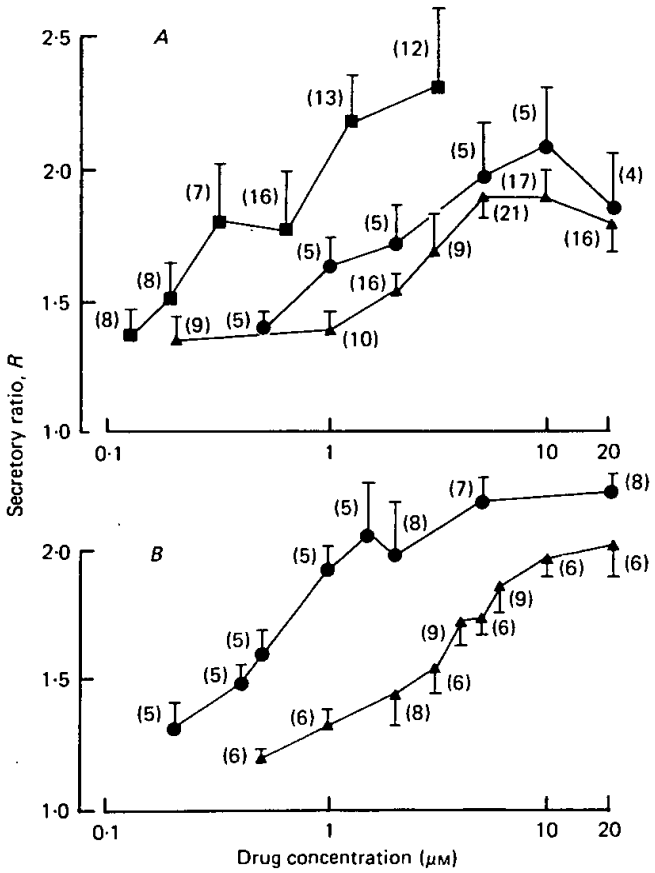

Fig. 1. Drug concentration on log scale plotted against secretory ratio. Points are mean values with S.E. bars, and numbers of observations are in parentheses. *A*, Isoprenaline (■), salmefamol (▲) and salbutamol (●). *B*, adrenaline (●) and noradrenaline (▲).

Adrenoceptor antagonists

Fig. 2 summarizes the effects of various antagonists on the responses to four of the adrenoceptor agonists. Each stomach was exposed to only one concentration of the agonists and was randomly allocated to either the test group containing the antagonist or to the control group. Not all possible agonist/antagonist pairs were studied and no antagonist studies were undertaken with salbutamol. The non-selective β-adrenoceptor antagonists caused inhibition of the response to all four agonists. The effects of the selective antagonists are less clear, with noradrenaline appearing to be consistently more susceptible to inhibition than the other agonists. For noradrenaline the control value of R was $1{\cdot}84 \pm 0{\cdot}07$ (S.E. of mean) ($3{\cdot}04 \pm 0{\cdot}32$ μmol H^+ cm^{-2} h^{-1}). In the presence of antagonists mean values of R ($\pm$S.E. of means) were: propranolol $1{\cdot}49 \pm 0{\cdot}09$ ($n = 8$) ($1{\cdot}82 \pm 0{\cdot}40$ μmol H^+ cm^{-2} h^{-1}), $2{\cdot}5 \times 10^{-5}$ M-butoxamine $1{\cdot}42 \pm 0{\cdot}05$ ($n = 13$) ($1{\cdot}41 \pm 0{\cdot}09$ μmol H^+ cm^{-2} h^{-1}) 10^{-4} M-practolol $1{\cdot}27 \pm 0{\cdot}07$ ($n = 6$) ($0{\cdot}68 \pm 0{\cdot}19$ μmole H^+ cm^{-2} h^{-1} and 10^{-4} M-phentolamine $1{\cdot}69 \pm 0{\cdot}09$ ($n = 8$) ($2{\cdot}43 \pm 0{\cdot}34$ μmol H^+ cm^{-2} h^{-1}). This inhibitory effect of butoxamine and practolol may be compared with their lack of effect against isoprenaline described in the

previous paper (Canfield & Price, 1981). This is dealt with in more detail in the Discussion.

Fig. 3 shows the log concentration–response curve for salmefamol and a two-point curve for isoprenaline in the presence of propranolol (2×10^{-5} M). The maximum response to salmefamol in the presence of propranolol occurred at 6×10^{-5} M where

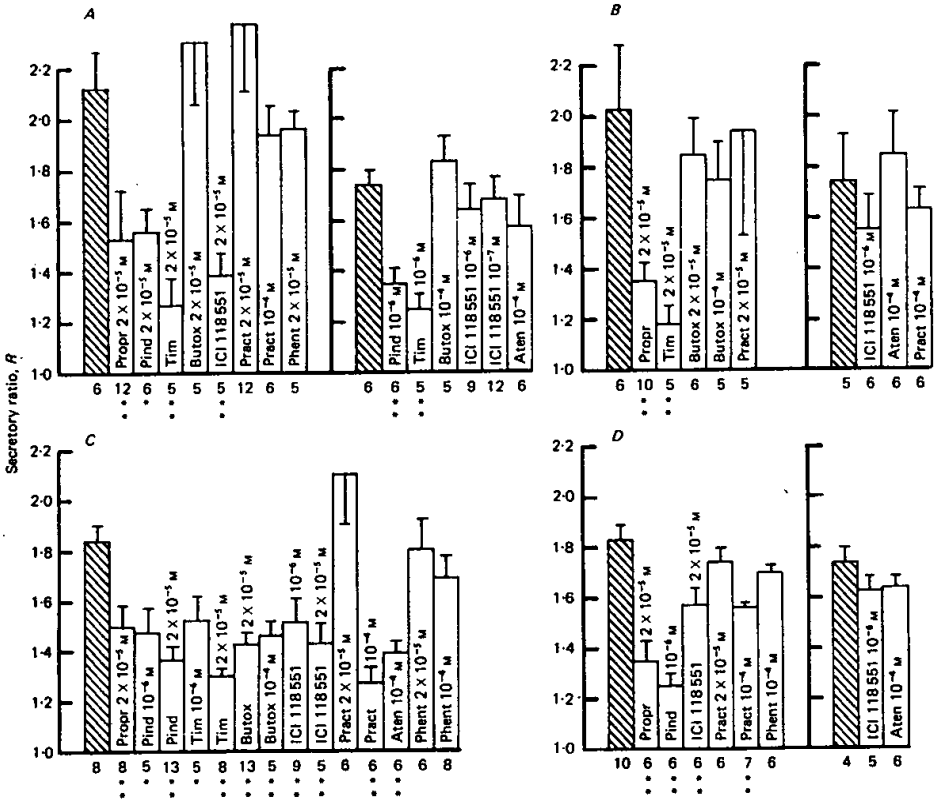

Fig. 2. The effect of various adrenoceptor antagonists on the secretory ratio obtained with four β-adrenoceptor agonists at a single submaximal concentration. Columns indicate mean values with S.E. bars, number of observations being shown at the base of each column. Statistically significant differences are shown by * = $P < 0.05$; ** = $P < 0.01$. Where agonist/antagonist interactions were studied in more than one series of experiments, the control values appropriate to each series are shown by the shaded columns. *A*, isoprenaline (1.25×10^{-6} M); *B*, salmefamol (5×10^{-6} M); *C*, noradrenaline (5×10^{-6} M); *D*, adrenaline (2×10^{-6} M). Aten, atenolol; Butox, butoxamine; Phent, phentolamine; Pind, pindolol; Pract, practolol; Propr, propranolol; Tim, timolol.

$R = 2.07 \pm 0.12$ (S.E. of mean, $n = 12$) (2.95 ± 0.28 μmol H^+ cm^{-2} h^{-1}). These results are consistent with a competitive antagonism of the response as there is no apparent change of slope and the maximum response to salmefamol is not reduced in the presence of propranolol. The α-adrenoceptor antagonist phentolamine at concentrations up to 10^{-4} M had no effect on the response to the β-adrenoceptor agonists.

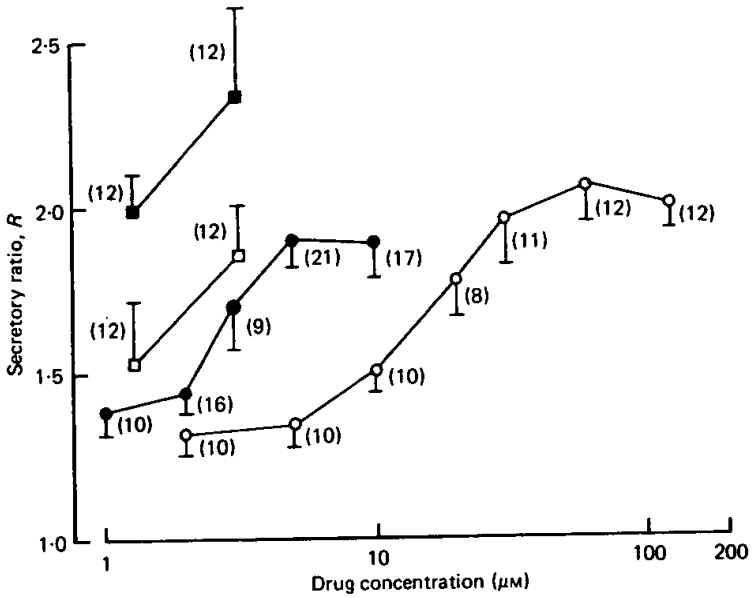

Fig. 3. The effect of propranolol (2×10^{-5} M, open symbols) on the response to isoprenaline (□) and salmefamol (○) compared with the response in the absence of antagonist (closed symbols). Points are mean values with S.E. bars, and numbers of observations are in parentheses.

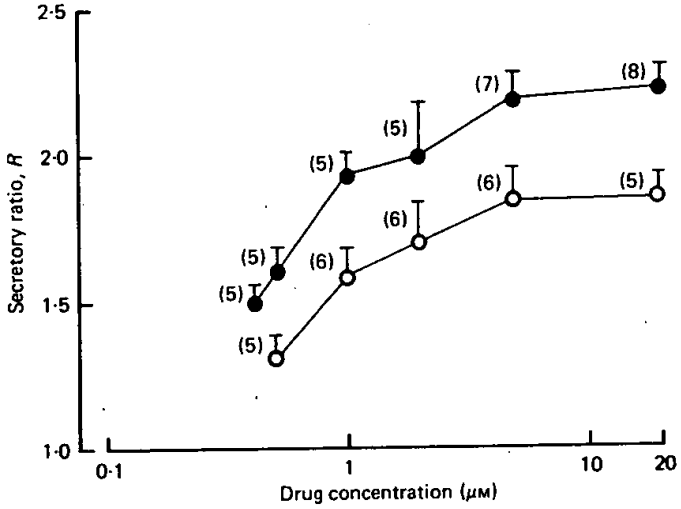

Fig. 4. Log adrenaline concentration plotted against secretory ratio for the immature stomach (●) and the adult mucosa preparation (○). Points are mean values with S.E. bars, and numbers of observations are in parentheses.

Adult mucosa

The rats used in this study were immature animals (age 15–20 days) and the sympathetic innervation of the gastro-intestinal tract is incomplete at this time (Yoshida, Taniyama & Tanaka, 1979). The whole stomach wall preparation also contains intact neural plexi. Both of these considerations might explain the results described above. We therefore undertook a few experiments with an isolated mucosa preparation from adult rats where the sympathetic innervation would be fully developed and the neural plexi substantially disrupted. The effects of adrenaline on the adult isolated mucosa are shown in Fig. 4 together with the original adrenaline curve from the immature rat preparations. The maximum R observed with adrenaline stimulation in the adult mucosa was $1{\cdot}85 \pm 0{\cdot}12$ (S.E. of mean, $n = 6$) ($1{\cdot}87 \pm 0{\cdot}50$ μmol H^+ cm^{-2} h^{-1}). Clearly adrenaline stimulated acid secretion in the adult preparation. Isoprenaline also stimulates this preparation (C. A. Price, personal communication). No further experiments were carried out with the adult mucosa as this preparation is 50 % thicker than the whole stomach wall from the immature rat, as judged from the blotted weights.

Other antagonists of acid secretion

β-Adrenoceptor agonists may stimulate acid secretion in our experiments by releasing one of the endogenous secretagogues. In the intact rat, adrenaline raises serum gastrin, for example (Hsu & Cooper, 1977). We have investigated this possibility by testing the effects of atropine, metiamide and prostaglandin E_2 using the same experimental design as for Fig. 2. The results are shown in Fig. 5.

Atropine (10^{-5} M) caused a significant inhibition of the response to bethanechol ($1{\cdot}7 \times 10^{-5}$ M) but had no effect on the sympathomimetic drugs. The mean control and test ratios (±S.E. of means) with bethanechol were $1{\cdot}89 \pm 0{\cdot}05$ ($n = 5$) ($4{\cdot}07 \pm 0{\cdot}96$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}10 \pm 0{\cdot}05$ ($n = 6$) ($0{\cdot}48 \pm 0{\cdot}25$ μmol H^+ cm^{-2} h^{-1}) and with noradrenaline $1{\cdot}83 \pm 0{\cdot}07$ ($n = 8$) ($3{\cdot}03 \pm 0{\cdot}32$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}69 \pm 0{\cdot}10$ ($n = 8$) ($3{\cdot}42 \pm 0{\cdot}30$ μmol H^+ cm^{-2} h^{-1}). Bunce, Parsons & Rollings (1976) reported that metiamide inhibited the response to gastrin in the rat isolated stomach and we anticipated that the use of this H_2 antagonist would enable us to exclude the possibility of β-adrenoceptor agonists releasing either histamine or gastrin. Metiamide (10^{-4} M) inhibited the response to histamine ($5{\cdot}4 \times 10^{-5}$ M) but was without effect on the β-adrenoceptor agonist responses or the response to pentagastrin ($2{\cdot}17 \times 10^{-7}$ M). The mean control and test ratios (±S.E. of means) for the action of metiamide on histamine were $1{\cdot}58 \pm 0{\cdot}11$ ($n = 5$) ($1{\cdot}56 \pm 0{\cdot}24$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}12 \pm 0{\cdot}05$ ($n = 6$) ($0{\cdot}22 \pm 0{\cdot}08$ μmol H^+ cm^{-2} h^{-1}). With pentagastrin corresponding values were $1{\cdot}66 \pm 0{\cdot}04$ ($n = 5$) ($2{\cdot}58 \pm 0{\cdot}16$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}68 \pm 0{\cdot}05$ ($n = 6$) ($1{\cdot}97 \pm 0{\cdot}36$ μmol H^+ cm^{-2} h^{-1}). Cimetidine ($1{\cdot}3 \times 10^{-4}$ M) was also without inhibitory action on pentagastrin-stimulated secretion (unpublished observation).

As a result of this finding we extended the study to include prostaglandin E_2, which significantly inhibited pentagastrin but not the responses to isoprenaline or adrenaline. The mean control and test ratios (±S.E. of means) for the action of prostaglandin E_2 on pentagastrin were $1{\cdot}66 \pm 0{\cdot}04$ ($n = 5$) ($2{\cdot}50 \pm 0{\cdot}68$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}36 \pm 0{\cdot}05$ ($n = 6$) ($1{\cdot}42 \pm 0{\cdot}40$ μmol H^+ cm^{-2} h^{-1}). Corresponding values for isopren-

aline were $2{\cdot}02 \pm 0{\cdot}18$ ($n = 5$) ($3{\cdot}33 \pm 0{\cdot}78$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}88 \pm 0{\cdot}08$ ($n = 5$) ($2{\cdot}5 \pm 0{\cdot}57$ μmol H^+ cm^{-2} h^{-1}). It is clear from these results that acid secretion stimulated by β-adrenoceptor agonists is unaffected by any of these three potent inhibitors of other established secretagogues.

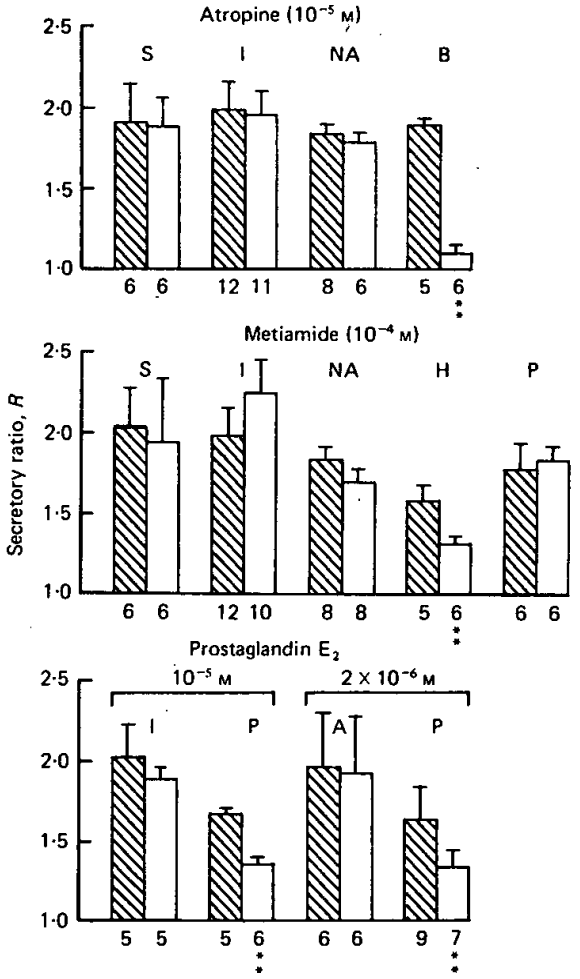

Fig. 5. The effects of atropine, metiamide and prostaglandin E_2 on the secretory ratios obtained with salmefamol (S, 5×10^{-6} M), isoprenaline (I, $1{\cdot}25 \times 10^{-6}$ M), noradrenaline (NA, 5×10^{-6} M), adrenaline (A, 2×10^{-6} M), bethanechol (B, $1{\cdot}7 \times 10^{-5}$ M), histamine (H, $5{\cdot}4 \times 10^{-5}$ M) and pentagastrin (P, $2{\cdot}2 \times 10^{-7}$ M). Columns are mean values with S.E. bars (the shaded columns being control values in the absence of antagonist), with numbers of observations at the base of each column.

DISCUSSION

The two naturally occurring catecholamines and the three synthetic β-adrenoceptor agonists all caused concentration-dependent increases in the rate of acid secretion by the immature rat stomach preparation. Both β_1-selective (noradrenaline) and β_2-selective (salmefamol, salbutamol) agonists stimulated acid output, suggesting that

both subtypes of receptors may be involved. This may partly explain the lower potency of these selective agonists compared with isoprenaline and adrenaline. The lack of inhibitory effect of phentolamine suggests that α-adrenoceptors are not involved in these responses. The response of the adult isolated mucosa to adrenaline and isoprenaline stimulation indicates that our results cannot be completely explained in terms of either the presence of intact neural plexi or immaturity of the preparation.

β-Adrenoceptor antagonists

The three non-selective antagonists (propranolol, pindolol and timolol) all significantly inhibited the response to β-adrenoceptor stimulation. The results with propranolol and salmefamol on isoprenaline (Fig. 3) were consistent with this being due to competitive inhibition.

The results with the selective β-adrenoceptor antagonists are more difficult to interpret. In both selective and non-selective cases, the concentration of antagonist required to produce significant inhibition is greater than might be expected from their respective pA_2 values. Recently Black and his colleagues (Angus & Black, 1979; Angus, Black & Stone, 1980) have drawn attention to the anomalous pA_2 values obtained with atropine and metiamide for inhibition of acid secretion in an isolated stomach preparation. They suggest that because of the act of secretion the antagonist concentration at the parietal cell never achieves equilibrium with the bathing medium and as a result anomalous values of pA_2 are obtained. This would presumably apply to all antagonist studies involving acid secretion *in vitro*, so that pA_2 values for drugs obtained on other tissues are of dubious value in the stomach. This would also mean that one cannot interpret the results obtained using selective antagonists with any certainty as the selectivity is not absolute but relative to the concentration of antagonist used. Thus, the β-selective antagonist ICI 118 551 at 10^{-5} M inhibited all four agonists but at this concentration is not likely to act selectively.

These considerations do not, however, explain the results with noradrenaline, where both the β_1- and β_2-selective antagonists inhibited the response at concentrations which were ineffective against the other agonists. The present experiments can provide no explanation for this for the reasons discussed above, and the whole question of the receptor type involved in these responses will probably require experiments using isolated parietal cell preparations where the problems discussed by Black *et al.* should no longer complicate the interpretation of the results.

Mechanism of action

The stimulatory effect of β-adrenoceptor agonists on acid secretion may be brought about by the release of some other secretagogue in the stomach. However, the lack of any inhibitory action of atropine, metiamide or prostaglandin E_2 on the adrenoceptor agonist responses indicates that they are not acting via release of the three established secretagogues: acetylcholine, histamine or gastrin. The simplest explanation of our findings is that there are β-adrenoceptors on the parietal cell which, when stimulated, bring about acid secretion. This explanation is supported by the recent finding that adrenaline over the same concentration range as used in this work caused a concentration-dependent increase in the cyclic AMP content of rat isolated parietal

cells (Günther, Wagner & Ruoff, 1980). In summary, our results confirm the findings of the preceding paper (Canfield & Price, 1981) that β-adrenoceptor agonists stimulate acid secretion *in vitro* by a β-receptor mechanism.

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